from spleen (DSL), 2) surveillance right ventricular endomyocardial biopsies (EMB) obtained at 1-week and 1-year after transplantation in 28-patients (age: recipient 55 ± 11.8; donor 32 ±7 years) who had a paired IVUS within 4-weeks and at one year after transplantation for the volumetric assessment of CAV. **Results:** AT_1 mRNA expression in DSL (median and 25th -75th percentile: 9.9, 3.1 - 23.8 folds relative to calibrator) correlated with that of EMB at 1-week (1.04, 0.6 - 2.0 folds; r = 0.60, P = 0.001) and at 1-yr (1.5, 0.6 - 3.8 folds; r = 0.66, P = 0.0003) after transplantation. mRNA expression of AT_1 in DSL (CMIT: r=0.73, P < 0.0001; CPV: r = 0.69, P < 0.0001) and EMB at 1-week (CMIT: r=0.52, P=0.005;CPV: r=0.56, P=0.002) and at 1-yr (CMIT: r=0.63, P < 0.0001; CPV: r=0.43, P=0.004) after transplantation were univariate predictors of CAV. Multivariate analysis showed that mRNA expression of AT_1 in DLS (P = 0.01) and in 1-year EMB (P = 0.03) as significant predictors of CAV with a combined r of 0.87 and R^2 of 0.75. AT_1 expression in DLS (7.4±9.8 vs 22.1±16.0 folds; P=0.004) and in EMBs at 1-year (1.3 ±1.6 vs 3.1±2.5 folds; P=0.01) were higher in recipients (n=13) demonstrating CAV as a more than 0.3mm CMIT from baseline to one year. **Conclusions:** mRNA expression of AT_1 in DSL correlate with that of early and one-year post transplant cardiac expression. Both DSL and cardiac AT_1 expression predict accelerated progression of CAV supporting a potentially important role of Ang II receptor blocker in retarding its progression after transplantation.

YOUNG INVESTIGATORS AWARDS COMPETITION

409 Young Investigators Awards Competition: Molecular and Cellular Cardiology

Monday, March 31, 2003, 2:00 p.m.-3:30 p.m.
McCormick Place, Room S104

2:00 p.m.

409-1 Local Delivery of Culture-Modified Mononuclear Cells Improves Endothelial Function and Attenuates Neointimal Formation in a Rabbit Balloon Injury Model

Rajiv Gulati, Dragan Jevremovic, Timothy E. Peterson, Tyra A. Witt, Laurel S. Kleppe, Cheryl S. Mueske, Amir Lerman, Richard G. Vile, Robert D. Simari, Mayo Clinic, Rochester, MN

Background: Bone marrow derived progenitor cells have been shown to contribute to endothelial replacement following vascular injury. In vitro culture of peripheral blood produces cells with phenotypic characteristics of endothelium. We hypothesized that autologous delivery of such culture-modified mononuclear cells (CMMC) to balloon injured arteries could beneficially modify the vascular response to injury.
Methods and Results: Rabbit peripheral blood mononuclear cells were cultured on fibronectin in endothelial growth media for 7 days yielding cells of endothelial lineage (CMMC). Continued culture resulted in highly proliferative outgrowth of cells with distinct endothelial phenotype (CD31, eNOS, acetylated LDL uptake). A rabbit model of balloon carotid injury was used to evaluate the effect of CMMC delivery on functional and structural vascular responses. Animals underwent balloon injury and immediate treatment with autologous CMMCs or saline control by 20 minutes of local dwelling or by systemic ear vein injection. Fluorescent labeled CMMCs were seen to incorporate into the vessel wall following both routes of delivery. Local CMMC administration at the time of balloon injury dramatically improved endothelial dependent vasoreactivity to acetylcholine (Ach) at 4 weeks compared with saline treatment (% max relaxation 77.6 +/- 6.4 vs 28.8 +/- 7 6, p<0.005; concentration Ach [-log M] to achieve 25% max relaxation 7.19 +/- 0.04 vs 5.38 +/- 0.06, p<0.005). CMMC treatment also significantly reduced neointimal formation at 4 weeks (int/med 0.39 +/- 0.08 vs 0.86 +/- 0.17, p<0.05).
Conclusions: These data demonstrate that delivery of CMMCs to balloon injured arteries is associated with markedly enhanced endothelial dependent vasoreactivity. Furthermore, CMMCs delivered at the time of injury significantly reduce subsequent neointimal formation.

2:15 p.m.

409-2 Antifibrotic Property of Brain Natriuretic Peptide in Cardiac Fibroblasts: Cross-Talk Action With Endothelin-1 and Tumor Necrosis Factor on the Induction of Matrix Metalloproteinases

Toshihiro Tsuruda, Guido Boerrigter, Brenda K. Huntley, Josh A. Noser, Alessandro Cataliotti, John C. Burnett, Jr., Mayo Clinic, Rochester, MN

Background: Cardiac fibroblasts (CFs) produce extracellular matrix (ECM) proteins and participate in the remodeling of the heart. Brain natriuretic peptide (BNP) is known to be activated in heart failure, and inhibit cellular proliferation; however, it is unknown if BNP participates in the degradation of ECM turnover. To understand the role of BNP as an anti-fibrotic factor in the progression of heart failure, we examined the effect of BNP and its signaling system on the activation of matrix metalloproteinases (MMPs), a key enzyme for the degradation of ECM proteins. In addition, we looked at the interactions between BNP and a fibrotic factor, endothelin-1 (ET-1), and a pro-inflammatory cytokine, TNF-alpha.
Methods: CFs isolated from normal adult canine ventricle were used. Techniques for zymographic gelatinase assay and Western blotting were employed to detect the gelatinase abundance and the protein levels for MMPs, respectively.
Results: One micro mol/L BNP significantly (p<0.01) enhanced zymographic gelatinase-A (MMP-2) abundance. In addition, protein expressions of MMP-1, -2, -3 and membranous type-1 MMP were significantly increased by BNP, while MMP-9 and MMP-13 were unchanged. The cGMP analogue 8-bromo-cGMP (10^{-4} mol/L) mimicked the BNP effect, whereas inhibition of protein kinase G (PKG) by KT5823 (10^{-6} mol/L) significantly (p<0.05) attenuated BNP-induced zymographic MMP-2 abundance. ET-1 (10^{-7} mol/L) down-regulated the zymographic MMP-2 abundance and BNP reversed the action of ET-1, while TNF-alpha (10^{-7} mol/L) increased BNP-induced zymographic MMP-2 abundance in a synergistic manner.
Conclusions: This study reports that BNP increases MMPs via cGMP-PKG signaling. In addition, cross-talk between BNP and ET-1, TNF-alpha results in different biological effects. These findings suggest that BNP participates in the remodeling of myocardial structure in the progression of heart failure via the control of cardiac fibroblast function.

2:30 p.m.

409-3 Identification of Differential Gene Expression Patterns in Patients With End-Stage Ischemic and Nonischemic Cardiomyopathies

Xinqiang Han, David Fermin, Jennifer Hall, Soon Park, Richard A. King, Leslie W. Miller, University of Minnesota Medical School, Minneapolis, MN

Background: End-stage cardiomyopathy (ESCM) is associated with altered expressions of multiple genes. Unloading by the left ventricular assist device (LVAD) which results in recovery of hemodynamic and cellular abnormalities may lead to further dynamic gene expression changes.
Methods: A microarray technique representing approximately 2/3 of known human genome (22283 genes, Affymetrix) was used to probe paired left ventricular samples from 15 patients (7 ischemic, 8 non-ischemic, on LVAD for 1 to 22 months) obtained at LVAD implant and transplantation; real-time polymerase chain reaction (RT-PCR) was used to further confirm selected gene transcript changes.
Results: At a p value of <0.01, at least 196 genes encompassing both cell structure protein/matrix and subcellular signaling proteins regulating inotropy, metabolism, myocardial hypertrophy and apoptosis were differentially expressed between ischemic and non-ischemic ESCM. In ischemic ESCM, LVAD support resulted in increase of 68 genes and decrease of 81 genes. In non-ischemic ESCM, LVAD support resulted in increase of 50 genes and decrease of 3 genes. Five genes (Ribosomal protein L4, Heterogeneous nuclear ribonucleoprotein, Glycine amidinotransferase, Aquaporin 7, and KIAA0713 protein) were found concordantly altered in both ischemic and non-ischemic ESCM. However, these five genes responded very differently to unloading by LVAD. When patients were grouped by the duration of LVAD support (Month: < 2, 2-10, >10), a greater number of genes were found altered with longer LVAD support and the majority of genes in each of the three duration groups were distinct. RT-PCR experiments using specific primers designed for natriuretic peptide precursor B, collagen type Ia, four and a half LIM domains 1, and myosin light chain 2a confirmed the microarray finding that the first two genes were down-regulated and the last two genes were up-regulated following LVAD.
Conclusion: Ischemic and non-ischemic ESCM are associated with dynamic and differential expressions of different genes. Remodeling following LVAD is likely an active process involving activation and inactivation of different functioning groups of genes in the two disease entities.

2:45 p.m.

409-4 Combinatorial Cytokine Gene Therapy Induces Synergistic Immunosuppression and Tolerance in Cardiac Allograft

Hiroshi Furukawa, Kiyohiro Oshima, Hyde Russell, Thomas Tung, Jun Xu, Guanggen Cui, Hillel Laks, Luyi Sen, UCLA, Los Angeles, CA

Background: Previous studies have shown that ex vivo interleukin-10 (IL-10) gene therapy suppressed alloimmune responses and prolonged allograft survival, but true tolerance was not achieved. We assessed the hypothesis that liposome-mediated ex vivo intracoronary interleukin-4 (IL-4) and IL-10 combined gene therapy may generate synergistic immunosuppression and induce allograft tolerance.
Methods: A functional cervical heterotopic heart transplant model of rabbits was used to evaluate the efficiency and efficacy of the gene therapy.
Results: The mean survival of cardiac allograft was significantly (p<0.05) prolonged from 7±1 days in Control Group (CG) to 28±7 days in IL-10 gene therapy Group (IL-10G) and 135±20 days in IL-4 and IL-10 combined gene therapy Group (IL-4&10G). The transgene and protein expression in IL-4&10G reached the peak in postoperative day (POD) 5-8, and slowly reduced thereafter. The rejection score in IL-4&10G was significantly lower (2.2±0.2, p<0.05) than that of CG (3.6±0.2) and IL-10G (2.7±0.3) in POD3-6, and 2.0±0.0 in POD>31. In IL-4&10G, total graft infiltrating cells was reduced 31% in POD7-10 and 72% in POD>31, and the percentage of CD3+ T cells was significantly decreased (42.7±3.4% in CG, 28.9±5.8% in IL-10G and 20.9±0.5% in IL-4&10G, p<0.01) in POD7-10. The percentage of CD4+ T cells was significantly (p<0.01) reduced from 28.9±3.3% in CG to 20.9±7.2% in IL-10G and 13.8±0.3% in IL-4&10G in POD7-10. The reduction of CD8+ T cells was even more remarkable (19.6±3.4% in CG, 14.1±3.0% in IL-10G, 7.8±0.3% in IL-4&10G in POD7-10, p<0.01). IL-4 and IL-10 expression was correlated with the reduction of the graft infiltrating CD3+ T cells and CD4+/CD8+ ratio (p<0.05), and inversely correlated with the rejection score (p<0.01). In IL-4&10G and IL-10G, the cytotoxic activity of infiltrating T cells in the allograft was greatly reduced (78±8%, and 69±7%, respectively).
Conclusion: The liposome-mediated ex vivo intracoronary IL-4 and IL-10 combined gene